Survival patterns under Costa Rican field conditions of the gregarious caterpillar *Euselasia chrysippe* (Lepidoptera: Riodinidae), a potential biological control agent of *Miconia calvescens* (Melastomataceae) in Hawaii

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Abstract. Survival of *Euselasia chrysippe*, a natural herbivore of *Miconia calvescens*, was investigated under field and laboratory conditions in Costa Rica as part of a biological control program in Hawaii. Following its introduction to Pacific islands, *M. calvescens* has become a dominant invasive species and the gregarious caterpillar of *E. chrysippe* has emerged as a promising biological control candidate. Under laboratory conditions, survivorship from egg to adult was 63%. *E. chrysippe* produced viable clutches in an experimental field plot. Similar clutch sizes occurred in both natural habitats and the experimental plot. Stage-specific life tables encompassing two years in the experimental plot indicate that larval survivorship from egg to the end of the sixth instar was about 10%. Egg parasitism was low in natural habitats and nil under experimental conditions. Larval group sizes were similar in natural and experimental field conditions, suggesting that survivorship is similar in both environments. During the coldest dry period no larvae survived past the fifth instar. Rainfall was a limiting factor for the survivorship of *E. chrysippe* in the experimental plot, but temperature appears to be the factor that would limit the effect of *E. chrysippe* on *M. calvescens* in Hawaii. Efforts should be invested in natural quarantine facilities to provide a testing ground for this species in target environments.

Keywords: Classical biological control, clutch size, *Euselasia chrysippe*, gregarious caterpillar, larval survivorship, *Miconia calvescens*, out-planting

INTRODUCTION

Miconia calvescens D.C. (Melastomataceae) is a dominant invasive species in Hawaii, Australia and some tropical oceanic islands of the South Pacific. It is native to the Americas, from southern Mexico to northern Argentina (Medeiros & Loope, 1997). Following its introduction into Hawaii as an ornamental in 1961, it invaded a wide variety of habitats, including agricultural landscapes as well as

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wet forests, and was declared a noxious weed in 1992 (Kaiser, 2006). Several methods have been tested for its eradication, including aerial spraying of herbicides and manual extraction, but the large seed banks (Loope, 1997; Medeiros & Loope, 1997) make these methods costly and ineffective. To protect native ecosystems on tropical Pacific islands the use of introduced biological control agents is an option, which could prove to be not only safer, but also more effective than chemical applications (Denslow & Johnson, 2006).

Selection of the best agents for biological control is critical for this kind of program to achieve success. Many programs have failed due to the lack of basic information on the environmental requirements of candidate species (Hokkanen, 1985; Wapshere *et al.*, 1989) and poor climatic adaptation is the main reason given for failure in classical biological control programs worldwide (Stiling, 1993). Due to underlying biophysical factors, climate influences the establishment and survival of candidate biological control agents (Hoelmer & Kirk, 2005).

Biological control agents should originate from areas having a similar climate to that of the intended area of release (Stiling, 1993). A program to search

for biological control agents of *Miconia calvescens* in Costa Rica at the University of Costa Rica (UCR) began in 1999, and since then more than 50 species of phytophagous insects have been collected from this plant species (Hanson *et al.*, 2009). Among them, *Euselasia chrysippe* (Lepidoptera: Riodinidae) emerged as a viable candidate (Johnson, 2009), but detailed knowledge of its biology is necessary to evaluate the suitability of this species as a potential biological control agent. *E. chrysippe* ranges from southern Mexico to Colombia (DeVries, 1997; Warren *et al.*, 2005) and occurs commonly from sea level to 1500 m. In Costa Rica it is found on both the Caribbean and Pacific slopes in primary and secondary rain forests (DeVries, 1997). *Miconia elata* (DeVries *et al.*, 1992), *M. calvescens* (DeVries, 1997), *M. trinervia*, *Conostegia rufescens* (Melastomataceae) (Janzen & Hallwachs, 2005), *Miconia impetiolaris*, *M. appendiculata*, *M. donaena* and *M. longifolia* (Nishida, 2010) have been reported as host plants for *E. chrysippe*. The caterpillars of *E. chrysippe* are processionary and synchronize their movement, feeding, resting and molting (DeVries, 1997; Allen, 2010; Nishida, 2010).

Classical biological control projects have shown that understanding the response of the biological agents to various environmental conditions increased the chances of success (Caltagirone, 1981). Larval mortality factors and general life history of *Euselasia* species have only been investigated in depth in Brazil, where several species are defoliators of *Eucalyptus* (Zanuncio *et al.*, 1990, 2009; Zanuncio *et al.*, 1995; Sousa *et al.*, 2010). The primary objectives of this study were to generate life tables for the pre-adult stages of *E. chrysippe* on *M. calvescens*, compile information on their environmental requirements, and identify the main sources and trends of pre-adult mortality in natural habitats and an experimental field plot. The out-planting (experimental field) strategy has been infrequently used in the context of biological control, but when it has been used, it has provided positive results (Balciunas *et al.*, 1994; Wilker *et al.*, 2000). The present study seeks to provide background information on the climatic suitability of *E. chrysippe* before its release in Hawaii.

MATERIALS AND METHODS

Field data and specimen collection

Between 2003 and 2005 egg clutches and larvae were collected from the following sites in Costa Rica: Laguna Hule (10°18'15" N, 84°12'23" W; 750 m), Lake Arenal (10°28'17" N, 84°46'11" W; 525 m), El Ángel-Cariblanco (10°15'44.2" N, 84°10'19" W; 750 m), La

Selva Biological Station (10°25'52" N, 84°00'12" W; 50 m), Vereh (9°47' N, 83°31' W; 1200 m), Jabillos (9°54' N, 83°37' W; 750 m), Jicotea (9°48'29" N, 83°31'23" W; 900 m), Hitoy Cerere Biological Station (9°40'19" N, 83°01'28" W; 100 m), Las Cruces Biological Station (8°47'28" N, 82°57'26" W; 1095 m), and Cerro Nara (9°30' N; 84°01'2 W; 1000 m). In the Holdridge life zone classification system (Holdridge, 1967) all sites in this study are either tropical wet or rain forests, from basal to lower montane elevational zones. Each site was visited between one and seven times, and the eggs and larvae were taken from *Miconia calvescens* trees or saplings. Not all individuals were collected, since some clutches and larvae were just photographed (using a Nikon Coolpix 4500 camera) for data on clutch and group sizes in their natural habitats. Causes of egg mortality in the field (predators, parasitoids or pathogens) were determined visually on site.

Larval rearing in the laboratory and the experimental plot

Clutches (attached to a piece of leaf) were placed in Petri dishes, larvae in plastic bags (on leaves), and transported to the laboratory at the UCR in San José (elevation: 1100 m). In the laboratory, egg clutches were left in the Petri dishes until eclosion. Larvae were placed on *M. calvescens* potted plants, one clutch per plant, and pots were placed in large plastic containers half full of water to prevent escape. After the larvae pupated on the plants, the pots were placed in a controlled environmental chamber (Model 518, Electro-Tech Systems: 23°C, 14:10 h D/L, 70% RH). Larvae were monitored daily; digital photographs were taken every day to determine the exact number of individuals. This procedure was also followed when eggs brought from the field hatched. Larval survivorship and instar duration were thus obtained for *E. chrysippe* under laboratory conditions. Laboratory temperature was recorded using a HOBO® datalogger.

Adults were released into the Leonel Oviedo Biological Reserve (experimental field plot) close to *M. calvescens* trees. Twenty *M. calvescens* trees were planted in the reserve between July 2002 and March 2003 to form a small patch. By September 2003 the average height, DBH and number of leaves of the saplings were 1.7m, 21mm, and 23 leaves, respectively. By November 2006 the trees had grown to 4.1m, 57mm and 76 leaves. The Leonel Oviedo Biological Reserve is a 0.75-hectare secondary forest fragment located in the middle of the UCR campus in San José (9°56'15"N; 84°03'00"W) completely immersed in an urban environment (Nishida *et al.*, 2009). This patch served as an experimental field, as neither *E. chrysippe*

nor *M. calvescens* are native to this part of the country. This area corresponds to Tropical Premontane Moist Forest (Holdridge, 1967), characterized by a dry season with little or no rain between December and April.

After adults had been released, daily surveys of *M. calvescens* trees in the experimental plot were carried out to determine when and where the egg clutches were laid. As in the laboratory, digital photographs were taken to quantify eggs per clutch, and after hatching photographs were taken of each larval group to count the number of larvae left and to determine the changes in larval stage. These data were used to calculate pre-adult mortality for each cohort (each individual egg clutch), for all stages. Sixth instar larvae abandon their plant and pupate, so it was impossible to determine pupal mortality in the experimental plot.

Survivorship of immature stages in the experimental plot

Time periods were established based on weather. The study extended for 20 months and encompassed two rainy and two dry seasons. The four periods were: 1. 8 September to 30 December 2003 (Rainy 2003); 2. 19 December 2003 to 29 March 2004 (Dry 2004); 3. 28 July to 15 November 2004 (Rainy 2004); and 4. 29 December 2004 to 2 April 2005 (Dry 2005). Developmental periods extended from the day an egg clutch was laid to the day the last larva died or left the *M. calvescens* tree. The duration of the egg stage was compared between periods using analysis of variance (ANOVA); in this and subsequent ANOVA tests a Tukey post-hoc test was used to localize differences between means. The same was done with maximum daily temperature and minimum daily temperature; total precipitation was also quantified for each period (obtained from a meteorological station 200m away from the experimental plot). Weather data from La Selva Biological Station (1987-2006) and Las Cruces Biological Station (1994-1998, 2005, 2006) (OTS 2012) were used to establish a temperature range for this butterfly species in the wild.

For larval survivorship, all data were converted to stage-specific survivorship (the proportion of each cohort that entered the population as an egg and survived through each instar). The rate of larval survivorship was compared between periods, performing log-log (base 10) regressions on the proportion that survived per stage versus stage (survivorship curves). The slope for each cohort was calculated (Hunter 2000) and the means of the slopes were compared between stages (Rainy 2003, Dry 2004, Rainy 2004, Dry 2005) with an ANOVA.

RESULTS

Egg clutch and larval group sizes of *Euselasia chrysippe* in the field and experimental plot

Between September 2003 and January 2005, 690 adult females and 570 adult males raised in the laboratory were released in the experimental plot (Fig. 1). There was a bias of 18.3 females (SD = 8.1; N = 29) compared with 15.8 males (SD = 8.4; N = 29) (t_p = 2.84; df = 29; P = 0.01). A total of 149 egg clutches were laid on the leaves of *Miconia calvescens* trees in the experimental plot. Most of the clutches were laid within 3 weeks, 25 days being the longest period between release and finding of a clutch. Monthly egg clutch production was correlated neither with adult release (r = 0.02; n = 19; P = 0.94; Fig. 1) nor precipitation (r = 0.07; n = 19; P = 0.79; Fig. 1).

In the field (10 sites in Costa Rica) the mean number of eggs per clutch was 62.7 (SD = 22.3, n = 84, range: 13 to 134) and followed a normal distribution (Shapiro-Wilk's test: SW-W = 0.98; P = 0.4; Fig. 2). In the experimental plot the mean was 67.2 (SD = 19.4, n = 158, range: 15 to 131), also fitting to a normal distribution (SW-W = 0.99; P = 0.25; Fig. 2). There was no significant difference in clutch sizes between field and experimental plot (t = -1.61; df = 240; P = 0.11). The condition of clutches in the field was in general good; fifteen healthy clutches were found and collected, another 61.5% (N = 78) had all empty eggs showing that larvae had eclosed successfully. Only 19.2% (N = 78) of the egg clutches had damaged
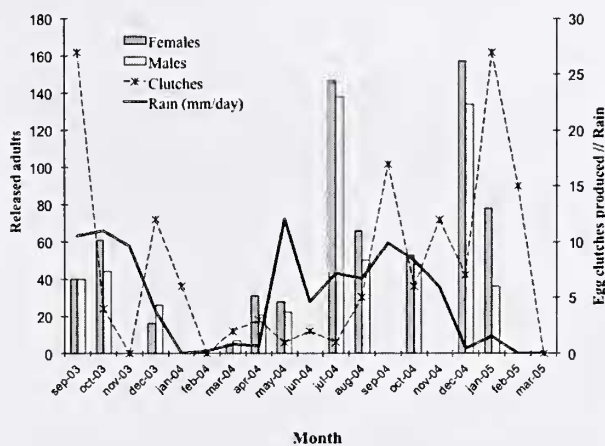

Figure 1. Release of adult *Euselasia chrysippe* and their egg clutch production in the Leonel Oviedo Biological Reserve (UCR; experimental field plot). The black line represents mean daily precipitation per month.

eggs, 11 (14.1%) of these had been attacked by parasitoids. There was no evidence of egg parasitoids in the experimental plot.

For some instars, a higher number of larvae per group were found in the field than in the experimental plot. There were more larvae per group in the second and third instars (Table 1), but not for the first, fourth and fifth instars; very few sixth instar groups were found in the field.

Survivorship of immature stages

In the experimental plot hatching time was longer in the Dry 2005 period (from 25 to 43 days) than in other periods ($F_{3,56}$ = 4.72; P = 0.005; Table 2). For the other three periods combined, the duration of the egg stage lasted between 21 and 37 days. Table 2 shows the comparison of environmental factors during all periods. The maximum daily temperature was lower during the Dry 2005 period ($F_{3,406}$ = 12.4; P < 0.0001), as was the minimum daily temperature ($F_{3,406}$ = 9.3; P < 0.0001). The annual pattern of daily temperature range in the experimental plot is similar to that found at Las Cruces BS, but much lower when compared to La Selva BS (Fig. 3). During the rainy periods rainfall was more than 300 mm/month, but less than 16mm/month during the dry periods (Fig. 1). In its natural habitats *M. calvescens* and *E. chrysippe* experience a maximum of one month without rain (Fig. 3).

The number of eggs per cohort did not differ between periods in the experimental plot ($F_{3,88}$ = 2.3; P = 0.90; Table 3). During the Rainy 2003 period there was a clutch where all eggs failed to hatch; in the Dry 2004 period two leaves with clutches attached dried out and fell. During the Dry 2005 period half of the cohorts were
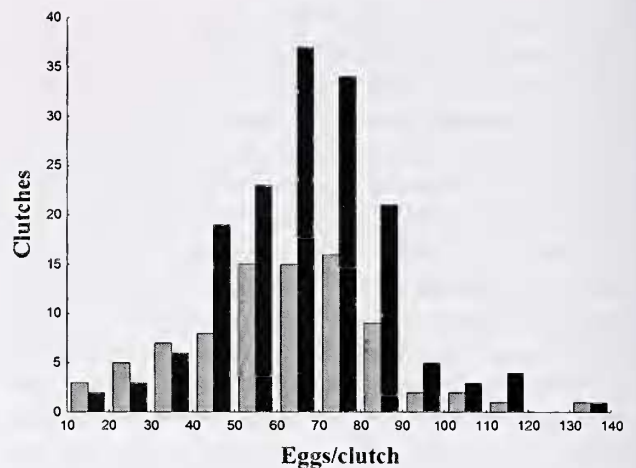

Figure 2. Number of eggs per clutch of *Euselasia chrysippe* found on *Miconia calvescens* trees. Black bars represent clutches found in the Leonel Oviedo Biological Reserve (experimental field plot) between October 2003 and December 2005; gray bars represent clutches found in the field (10 sites in Costa Rica) between December 2003 and December 2005.
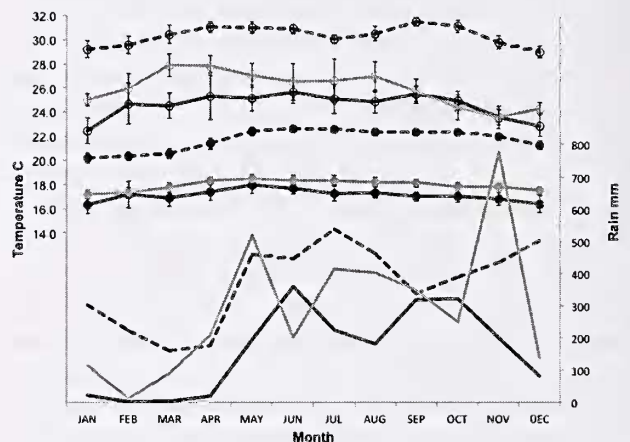

Figure 3. Mean (± SD) monthly minimum (full circles) and maximum (empty circles) temperatures (°C; left Y-axis), and total monthly precipitation (no circles; right Y-axis), for two sites in Costa Rica where *Miconia calvescens* grows naturally and the Leonel Oviedo Biological Reserve (experimental field plot – black continues line). La Selva BS (black discontinued line) is the hottest and Las Cruces BS (gray line) is the coldest (and driest) sites where *M. calvescens* and *Euselasia chrysippe* occur together in Costa Rica.

Table 1. Mean (±SD) number of *Euselasia chrysippe* larvae per group by instar, found on *Miconia calvescens* trees in the field (10 sites in Costa Rica) between December 2003 and August 2005, and in the Leonel Oviedo Biological Reserve (experimental field plot) between October 2003 and April 2005.

Instar	Field	n	Reserve	n	t	P
First	42.4 ± 22.5	12	37.2 ± 20.1	68	0.82	0.42
Second	47.1 ± 20.9	20	34.9 ± 21.0	39	2.11	0.039
Third	43.6 ± 22.7	15	29.5 ± 14.7	34	2.6	0.01
Fourth	29.7 ± 18.6	9	27.6 ± 13.8	29	0.36	0.72
Fifth	22.5 ± 31.4	6	24.0 ± 13.8	25	0.07	0.94
Sixth	7.0 ± 8.5	2	21.4 ± 14.1	19	[a]	

[a] test was not performed due to small sample size.

lost during the egg stage (Table 3): five fell with leaves, three were preyed upon by ants, three did not hatch, in two cases the larvae hatched but died next to the egg shells (with no evidence of predation), and in three cases the larvae hatched but disappeared without feeding.

It was more likely for early instar larvae to die during the dry periods than during the rainy periods ($F_{3,88} = 7.91$; $P < 0.0001$; Fig. 4). The number of cohorts ($X^2 = 3.51$; df = 2; $P = 0.17$) and larvae per cohort ($F_{2,16} = 0.54$; $P = 0.59$) did not differ between the Rainy 2003, Dry 2004 and Rainy 2004 periods (Table 3), through the end of the sixth instar. All fifth instar larvae died during the Dry 2005 period (Fig. 4). For the other three periods larval survivorship to the end of the sixth instar was about 10%. Mortality due to egg predation (by ants) occurred during all periods, but overall predation rates could not be quantified under experimental conditions or in their natural habitats.

The number of survivors in the laboratory decreased slowly and constantly until the end of the sixth instar (Fig. 4), reaching 63.1% (SD = 7.1, N = 7) from egg to adult. No cohort reached 100% mortality in the laboratory. The duration of the entire larval period was shorter in the laboratory, with a mean of 36.5 days (SD = 3.8), compared to 46.6 days (SD = 5.0) in the experimental plot (t = 4.9; df = 24; $P < 0.0001$). Mean temperature was higher in the laboratory than in the experimental plot, 22.2°C (SD = 1.0) versus 20.2°C (SD = 1.2) (t = -5.5; df = 260; $P < 0.0001$).
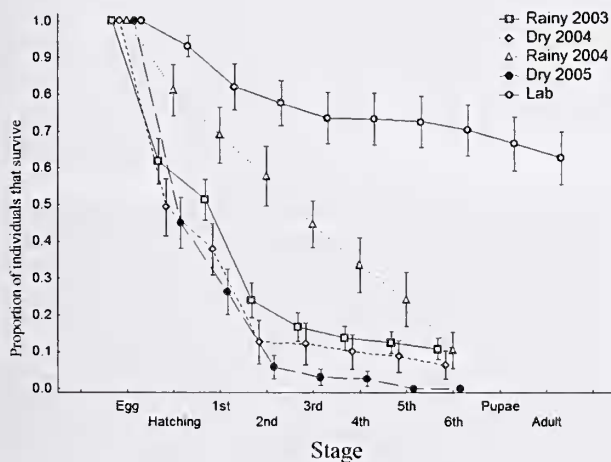

Figure 4. Survivorship (Mean ± SE) of *Euselasia chrysippe* individuals on *Miconia calvescens* trees in the Leonel Oviedo Biological Reserve (experimental field plot) during four time periods and in the laboratory (UCR). Slope (Mean ± SE) of the regression between survivorship and larval stage: -0.53 ± 0.12[ab] (Rainy 2003; n = 26), -0.80 ± 0.15[bc] (Dry 2004; n = 19), -0.26 ± 0.06[a] (Rainy 2004; n = 16) and -1.10 ± 0.12[c] (Dry 2005; n = 31). Means followed by the same letter are not significantly different (P > 0.05, Tukey test).

DISCUSSION

Euselasia chrysippe survived successfully in an experimental field plot, under less than ideal biotic and abiotic conditions. Adult females reared in the laboratory mated and laid eggs on *Miconia calvescens* trees, even though the latter were smaller than in natural habitats (Badenes-Pérez *et al.*, 2010). Egg clutch and larval group sizes of *E. chrysippe* found under experimental conditions reflected what was found in the field. A general larval survivorship (experimental plot) of 10% to the pupal stage agrees with reports for other gregarious species of Lepidoptera and Symphyta (Hunter, 2000). Survivorship was lower in the first two larval instars, especially during the dryer periods, which is also common in other Lepidoptera (Zalucki *et al.*, 2002).

Egg parasitism of *E. chrysippe* was low in the field and similar to that found by Nishida (2010), much lower than the parasitism rate for *E. eucerus* eggs in Brazil (Zanuncio *et al.*, 2009). The lack of evidence for parasitoid mortality in the experimental plot probably reflects the isolated location of this small patch of secondary forest. The closest fragment of forest is 4.3 km away, and the closest old growth forest is 12 km away (Braulio Carrillo National Park). An egg parasitoid of *E. chrysippe*, *Encarsia porteri* (Aphelinidae), has been reared in its native range (Nishida, 2010). Although many *Encarsia* species have been introduced to Hawaii to control whiteflies (Heu *et al.*, 2004), *E. porteri* is not among them (Bishop Museum, 2002). This has major implications for the introduction of *E. chrysippe* in Hawaii, as it reduces the risk of egg parasitism should it be released as a biological control agent of *M. calvescens*. Larval group sizes observed in the field versus the experimental plot remained similar until the end of the fifth instar. Few sixth instar groups were found in the field, and these groups were small compared to the ones in the experimental plot. One likely mortality factor acting on these older larvae in the field are tachinid parasitoids, as reported by Janzen & Hallwachs (2005) and Nishida (2010). Larval parasitism and predation were not quantified in this study, but predation events were commonly observed in all larval stages in the experimental plot.

Low precipitation and temperatures were correlated with high mortality and local extinction of immature stages of *E. chrysippe* under experimental field conditions. This long dry season can be considered extreme for this species, since in its native range in Costa Rica there is typically a mean rainfall of 280-600 mm/month (Fig. 3; Allen, 2007) and a maximum of one dry month. The absence of rain

Table 2. Duration (Mean ± SD) of the egg stage for *Euselasia chrysippe* during four time periods in the Leonel Oviedo Biological Reserve (experimental field plot), and the recorded environmental factors associated with each period.

Variable	Rainy 2003	Dry 2004	Rainy 2004	Dry 2005
n (egg clutches)	23	13	7	17
Duration of egg stage (days)	30.1 ± 3.5[a]	29.8 ± 4.3[a]	29.0 ± 2.3[a]	34.1 ± 5.0[b]
Environmental factor				
Maximum Daily Temperature (°C)	24.8 ± 1.8[a]	24.4 ± 2.6[a]	25.0 ± 1.5[a]	23.2 ± 2.8[b]
MaxDT range (°C)	19.0-28.1	19.8-30.9	19.9-27.9	17.8-29.9
Minimum Daily Temperature (°C)	17.0 ± 0.8[a]	17.3 ± 1.8[a]	17.0 ± 0.8[a]	16.4 ± 1.4[b]
MinDT range (°C)	15.0-18.8	13.0-22.6	14.5-19.1	13.3-19.0
n (days)	102	102	111	95

Means followed by the same letter within a line are not significantly different ($P > 0.05$, Tukey test).

Table 3. Partial life table for the immature stages of *Euselasia chrysippe* on *Miconia calvescens* in the Leonel Oviedo Biological Reserve (experimental field plot) during four time periods. Numbers represent quantity of cohorts that had living individuals at the end of each stage.

Variable	Rainy 2003	Dry 2004	Rainy 2004	Dry 2005
Clutches (cohorts)	26	19	16	31
Eggs/cohort[a]	67.3 ± 23.4	62.4 ± 11.3	64.8 ± 14.0	65.3 ± 23.3
1st instar (cohorts)	23	15	15	15
2nd instar	16	4	15	4
3rd instar	13	4	15	4
4th instar	13	4	10	2
5th instar	13	4	8	.
6th instar[b]	12	3	4	.
Larvae/cohort 6th instar[a]	19.0 ± 15.2	28.3 ± 17.9	23.3 ± 8.1	.

[a] Mean ± SD, there is no difference between periods ($P > 0.05$, ANOVA); [b] there is no difference between periods ($P > 0.05$, X^2).

for more than four months in the experimental plot caused *Miconia calvescens* leaves to dry out and fall from the trees, causing the immediate loss of many egg clutches. The areas of *M. calvescens* infestations in Hawaii do not suffer from a dry season longer than a month (Giambelluca *et. al.*, 1986), thus probably reducing to a minimum this kind of mortality (T. Johnson, pers. comm.). Daily temperature range in the experimental plot is similar to that at Las Cruces BS, the coldest site where both *M. calvescens* and *E. chrysippe* occur in Costa Rica (Allen, 2007); however, during the Dry 2005 period the daily temperature was at least 1°C lower than during the Dry 2004 period. This could explain the zero survivorship during the coldest dry period, as it seems that a mean minimum daily temperature of 17.0°C is the lower thermal limit for *E. chrysippe* in natural habitats (Fig. 3). If introduced to Hawaii, *E. chrysippe* would probably not

be able to establish in all areas where *M. calvescens* is invasive, as this plant has invaded areas with minimum daily temperature reaching 10°C in both Hawaii (Allen, 2007) and Tahiti (Meyer, 1998). Eggs took longer to hatch during the coldest dry season in the experimental plot. It remains to be seen if this had a negative effect on larval survivorship, but eggs were definitely exposed longer to predators, leaf fall and also suffered an increased risk of dehydration and depletion of food reserves (Holliday, 1985).

This study shows that larval survivorship of *Euselasia chrysippe* in an experimental field plot is similar to that in natural habitats and the chief limiting factor for pre-adult survivorship is a four-month dry season. The areas of *M. calvescens* infestations in Hawaii have a favorable rainfall of 150-300mm/month (Giambelluca *et. al.*, 1986; Juvic & Juvic, 1998; Reichert *et al.*, 2010), with no dry season longer than a month.

With respect to temperature, the areas most affected by *M. calvescens* fall into the range tolerated by *E. chrysippe* (Allen, 2007; Kaiser, 2006), but some of the less affected are too cold. This might be a non-issue since early detection of *M. calvescens* in the island of Kauai (Kauai Invasive Species Committee), which is too cold for *E. chrysippe*, has prevented its expansion and there might not be any trees of reproductive age on the island (Conant & Nagai, 1998). These results are encouraging for the possible introduction of *E. chrysippe* to Hawaii as a biological control agent of *Miconia calvescens*.

The current levels of scientific review have notably increased the safety of biological control programs in Hawaii since 1975 (Reimer, 2002); no purposely introduced species approved for release have been recorded to attack native or other desirable species in the last 37 years (Funasaki *et al.*, 1988; Reimer, 2002). There is thus good precedent for continuing this process with *E. chrysippe*. The present study provides essential data on general survivorship patterns and climate suitability; the next steps will be to quantify the impact of predation and parasitism on the larval stages, examine host specificity in greater detail, and determine the probability of in-situ establishment. Probable biotic sources of mortality of *E. chrysippe* larvae in Hawaii include generalist predators (Johnson, 2009) such as introduced vespid wasps and ants (Gambino *et al.*, 1987; Reimer, 1994). Finally, efforts should be made to provide adequate space for butterfly mating in the quarantine facilities in Hawaii, and field cage trials to provide suitable testing grounds for this species in target environments.

Acknowledgements

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